

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information therefore already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs (4-17). The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs by their very nature lend themselves to a third containment mechanism--namely, the application of highly specific biological barriers. In fact, natural barriers do exist which either limit the infectivity of a vector or vehicle (plasmid, bacteriophage or virus) to specific hosts, or its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying different combinations of the physical and biological barriers to a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the guidelines for research on the different kinds of recombinant DNAs (Section III).

A. Standard practices and training - The first principle of containment is a strict adherence to good microbiological practices (4-13). Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. This should include at least training in aseptic techniques and instruction in the biology of the organisms used in the experiments so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard should have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the work and the emergency plan. If a research group is working with a known pathogen for which an effective vaccine is available, all workers should be immunized. Serological monitoring, where appropriate, should be provided.

B. Physical containment levels - A variety of combinations (levels) of special practices, equipment, and laboratory installations that provide additional physical barriers can be formed. For example, 31 combinations are listed in "Laboratory Safety at the Center for Disease Control" (4); four levels are associated with the "Classification of Etiologic Agents on the Basis of Hazard" (5), four levels were recommended in the "Summary Statement of the Asilomar Conference on Recombinant DNA Molecules" (3); and the National Cancer Institute uses three levels for research on oncogenic viruses (6). We emphasize that these are an aid to, and not a substitute for, good technique. Personnel must be competent in the effective use of all

equipment needed for the required containment level as described below. We define only four levels of physical containment here, both because the accuracy with which one can presently assess the biohazards that may result from recombinant DNAs does not warrant a more detailed classification, and because additional flexibility can be obtained by combination of the physical with the biological barriers. Though different in detail, these four levels (P1 < P2 < P3 < P4) approximate those given for human etiologic agents by the Center for Disease Control (i.e., classes 1 through 4; ref. 5), in the Asilomar summary statement (i.e., minimal, low, moderate, and high; ref. 3), and by the National Cancer Institute for oncogenic viruses (i.e., low, moderate, and high; ref. 6), as is indicated by the P-number or adjective in the following headings. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of hazardous organisms.

We anticipate, and indeed already know of, procedures (14) which enhance physical containment capability in novel ways. For example, miniaturization of screening, handling, and analytical procedures provides substantial containment of a given host-vector system. Thus, such procedures should reduce the need for the standard types of physical containment, and such innovations will be considered by the Recombinant DNA Molecule Program Advisory Committee.

The special practices, equipment and facility installations indicated for each level of physical containment are required for the safety of laboratory workers, other persons, and for the protection of the environment. Optional items have been excluded; only those items deemed absolutely necessary for safety are presented. Thus, the listed requirements present basic safety

criteria for each level of physical containment. Other microbiological practices and laboratory techniques which promote safety are to be encouraged. Additional information giving further guidance on physical containment is provided in a supplement to the guidelines (Appendix D).

P1 Level (Minimal) - A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P1 level is a laboratory that possesses no special engineering design features. It is a laboratory commonly used for microorganisms of no or minimal biohazard under ordinary conditions of handling. Work in this laboratory is generally conducted on open bench tops. Special containment equipment is neither required nor generally available in this laboratory. The laboratory is not separated from the general traffic patterns of the building. Public access is permitted.

The control of biohazards at the P1 level is provided by standard microbiological practices of which the following are examples: (i) Laboratory doors should be kept closed while experiments are in progress. (ii) Work surfaces should be decontaminated daily and following spills of recombinant DNA materials. (iii) Liquid wastes containing recombinant DNA materials should be decontaminated before disposal. (iv) Solid wastes contaminated with recombinant DNA materials should be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. (v) Although pipetting by mouth is permitted, it is preferable that mechanical pipetting devices be used. When pipetting by mouth, cotton-plugged pipettes shall be employed. (vi) Eating, drinking, smoking, and storage of food in the working area should be discouraged. (vii) Facilities to wash hands should

be available. (viii) An insect and rodent control program should be provided. (ix) The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

P2 Level (Low) - A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P2 level is similar in construction and design to the P1 laboratory. The P2 laboratory must have access to an autoclave within the building; it may have a Biological Safety Cabinet.¹ Work which does not produce a considerable aerosol is conducted on the open bench. Although this laboratory is not separated from the general traffic patterns of the building, access to the laboratory is limited when experiments requiring P2 level physical containment are being conducted. Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

The P2 laboratory is commonly used for experiments involving microorganisms of low biohazard such as those which have been classified by the Center for Disease Control as Class 2 agents (5).

The following practices shall apply to all experiments requiring P2 level physical containment: (i) Laboratory doors shall be kept closed while experiments are in progress. (ii) Only persons who have been advised of the potential biohazard shall enter the laboratory. (iii) Children under 12 years of age shall not enter the laboratory. (iv) Work surfaces shall be decontaminated daily and immediately following spills of recombinant DNA materials. (v) Liquid wastes of recombinant DNA materials shall be decontaminated before disposal. (vi) Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof

container before removal from the laboratory. Packaged materials shall be disposed of by incineration or sterilized before disposal by other methods. Contaminated materials that are to be processed and reused (i.e., glassware) shall be decontaminated before removal from the laboratory. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the working area. (ix) Facilities to wash hands shall be available within the laboratory. Persons handling recombinant DNA materials should be encouraged to wash their hands frequently and when they leave the laboratory. (x) An insect and rodent control program shall be provided. (xi) The use of laboratory gowns, coats, or uniforms is required. Such clothing shall not be worn to the lunch room or outside the building. (xii) Animals not related to the experiment shall not be permitted in the laboratory. (xiii) Biological Safety Cabinets¹ and/or other physical containment equipment shall be used to minimize the hazard of aerosolization of recombinant DNA materials from operations or devices that produce a considerable aerosol (e.g., blender, lyophilizer, sonicator, shaking machine, etc.). (xiv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

P3 Level (Moderate) - A laboratory suitable for experiments involving recombinant DNA molecules requiring physical containment at the P3 level has special engineering design features and physical containment equipment. The laboratory is separated from areas which are open to the general public. Separation is generally achieved by controlled access corridors, air locks, locker rooms or other double-doored facilities which are not available for use by the general public. Access to the laboratory is controlled. Biological

Safety Cabinets¹ are available within the controlled laboratory area. An autoclave shall be available within the building and preferably within the controlled laboratory area. The surfaces of walls, floors, bench tops, and ceilings are easily cleanable to facilitate housekeeping and space decontamination.

Directional air flow is provided within the controlled laboratory area. The ventilation system is balanced to provide for an inflow of supply air from the access corridor into the laboratory. The general exhaust air from the laboratory is discharged outdoors and so dispersed to the atmosphere as to prevent reentry into the building. No recirculation of the exhaust air shall be permitted without appropriate treatment.

No work in open vessels involving hosts or vectors containing recombinant DNA molecules requiring P3 physical containment is conducted on the open bench. All such procedures are confined to Biological Safety Cabinets.¹

The following practices shall apply to all experiments requiring P3 level physical containment: (i) The universal biohazard sign is required on all laboratory access doors. Only persons whose entry into the laboratory is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards before entry and they shall comply with posted entry and exit procedures. Children under 12 years of age shall not enter the laboratory. (ii) Laboratory doors shall be kept closed while experiments are in progress. (iii) Biological Safety Cabinets¹ and other physical containment equipment shall be used for all procedures that produce aerosols of recombinant DNA materials (e.g., pipetting,

plating, flaming, transfer operations, grinding, blending, drying, sonicating, shaking, etc.). (iv) The work surfaces of Biological Safety Cabinets¹ and other equipment shall be decontaminated following the completion of the experimental activity contained within them. (v) Liquid wastes containing recombinant DNA materials shall be decontaminated before disposal. Solid wastes contaminated with recombinant DNA materials shall be decontaminated or packaged in a durable leak-proof container before removal from the laboratory. Packaged material shall be sterilized before disposal. Contaminated materials that are to be processed and reused (i.e., glassware) shall be sterilized in the controlled laboratory area or placed in a durable leak-proof container before removal from the controlled laboratory area. This container shall be sterilized before the materials are processed. (vii) Pipetting by mouth is prohibited; mechanical pipetting devices shall be used. (viii) Eating, drinking, smoking, and storage of food are not permitted in the laboratory. (ix) Facilities to wash hands shall be available within the laboratory. Persons shall wash hands after experiments involving recombinant DNA materials and before leaving the laboratory. (x) An insect and rodent control program shall be provided. (xi) Laboratory clothing that protects street clothing (i.e., long sleeve solid-front or wrap-around gowns, no-button or slipover jackets, etc.) shall be worn in the laboratory. FRONT-BUTTON LABORATORY COATS ARE UNSUITABLE. Gloves shall be worn when handling recombinant DNA materials. Provision for laboratory shoes is recommended. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry. (xii) Raincoats, overcoats, topcoats, coats, hats, caps, and such street outerwear shall not be kept in the laboratory.

(xiii) Animals and plants not related to the experiment shall not be permitted in the laboratory. (xiv) Vacuum lines shall be protected by filters and liquid traps. (xv) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xvi) If experiments of lesser biohazard potential are to be conducted in the same laboratory concurrently with experiments requiring P3 level physical containment they shall be conducted only in accordance with all P3 level requirements. (xvii) Experiments requiring P3 level physical containment can be conducted in laboratories where the directional air flow and general exhaust air conditions described above cannot be achieved, provided that this work is conducted in accordance with all other requirements listed and is contained in a Biological Safety Cabinet¹ with attached glove ports and gloves. All materials before removal from the Biological Safety Cabinet¹ shall be sterilized or transferred to a non-breakable, sealed container, which is then removed from the cabinet through a chemical decontamination tank, autoclave, ultraviolet air lock, or after the entire cabinet has been decontaminated.

P4 Level (High) - Experiments involving recombinant DNA molecules requiring physical containment at the P4 level shall be confined to work areas in a facility of the type designed to contain microorganisms that are extremely hazardous to man or may cause serious epidemic disease. The facility is either a separate building or it is a controlled area, within a building, which is completely isolated from all other areas of the building. Access to the facility is under strict control. A specific facility operations manual is available. Class III Biological Safety Cabinets¹ are available within work areas of the facility.

A P4 facility has engineering features which are designed to prevent the escape of microorganisms to the environment (14, 15, 16, 17). These features

include: (i) monolithic walls, floors, and ceilings in which all penetrations such as for air ducts, electrical conduits, and utility pipes are sealed to assure the physical isolation of the work area and to facilitate housekeeping and space decontamination; (ii) air locks through which supplies and materials can be brought safely into the facility; (iii) contiguous clothing change and shower rooms through which personnel enter into and exit from the facility; (iv) double-door autoclaves to sterilize and safely remove wastes and other materials from the facility; (v) a biowaste treatment system to sterilize liquid effluents if facility drains are installed; (vi) a separate ventilation system which maintains negative air pressures and directional air flow within the facility; and (vii) a treatment system to decontaminate exhaust air before it is dispersed to the atmosphere. A central vacuum utility system is not encouraged; if one is installed, each branch line leading to a laboratory shall be protected by a high efficiency particulate air filter.

The following practices shall apply to all experiments requiring P4 level physical containment: (i) The universal biohazard sign is required on all facility access doors and all interior doors to individual laboratory rooms where experiments are conducted. Only persons whose entry into the facility or individual laboratory rooms is required on the basis of program or support needs shall be authorized to enter. Such persons shall be advised of the potential biohazards and instructed as to the appropriate safeguards to ensure their safety before entry. Such persons shall comply with the instructions and all other posted entry and exit procedures. Under no condition shall children under 15 years of age be allowed entry. (ii) Personnel shall enter into and exit from the facility only through the clothing change and shower

rooms. Personnel shall shower at each exit from the facility. The air locks shall not be used for personnel entry or exit except for emergencies.

(iii) Street clothing shall be removed in the outer facility side of the clothing change area and kept there. Complete laboratory clothing including undergarments, pants and shirts or jumpsuits, shoes, head cover, and gloves shall be provided and used by all persons who enter into the facility. Upon exit, this clothing shall be stored in lockers provided for this purpose or discarded into collection hampers before personnel enter into the shower area.

(iv) Supplies and materials to be taken into the facility shall be placed in an entry air lock. After the outer door (opening to the corridor outside of facility) has been secured, personnel occupying the facility shall retrieve the supplies and materials by opening the interior air lock door. This door shall be secured after supplies and materials are brought into the facility.

(v) Doors to laboratory rooms within the facility shall be kept closed while experiments are in progress. (vi) Experimental procedures requiring P4 level physical containment shall be confined to Class III Biological Safety Cabinets.¹ All materials, before removal from these cabinets, shall be sterilized or transferred to a non-breakable sealed container, which is then removed from the system through a chemical decontaminated tank, autoclave, or after the entire system has been decontaminated. (vii) No materials shall be removed from the facility unless they have been sterilized or decontaminated in a manner to prevent the release of agents requiring P4 physical containment. All wastes and other materials and equipment not damaged by high temperature or steam shall be sterilized in the double-door autoclave. Biological materials to be removed from the facility shall be transferred to a non-breakable sealed

container which is then removed from the facility through a chemical decontamination tank or a chamber designed for gas sterilization. Other materials which may be damaged by temperature or steam shall be sterilized by gaseous or vapor methods in an air lock or chamber designed for this purpose. (viii) Eating, drinking, smoking, and storage of food are not permitted in the facility. Foot-operated water fountains located in the facility corridors are permitted. Separate potable water piping shall be provided for these water fountains. (ix) Facilities to wash hands shall be available within the facility. Persons shall wash hands after experiments. (x) An insect and rodent control program shall be provided. (xi) Animals and plants not related to the experiment shall not be permitted in the facility. (xii) If a central vacuum system is provided, each vacuum outlet shall be protected by a filter and liquid trap in addition to the branch line HEPA filter mentioned above. (xiii) Use of the hypodermic needle and syringe shall be avoided when alternate methods are available. (xiv) If experiments of lesser biohazard potential are to be conducted in the facility concurrently with experiments requiring P4 level containment, they shall be confined in Class I or Class II Biological Safety Cabinets¹ or isolated by other physical containment equipment. Work surfaces of Biological Safety Cabinets¹ and other equipment shall be decontaminated following the completion of the experimental activity contained within them. Mechanical pipetting devices shall be used. All other practices listed above with the exception of (vi) shall apply.

C. Shipment - To protect product, personnel, and the environment, all recombinant DNA material will be shipped in containers that meet the

requirements issued by the U.S. Public Health Service (Section 72.25 of Part 72, Title 42, Code of Federal Regulations), Department of Transportation (Section 173.387 (b) of Part 173, Title 49, Code of Federal Regulations) and the Civil Aeronautics Board (C.A.B. No. 82, Official Air Transport Restricted Articles Tariff No. 6-D) for shipment of etiologic agents. Labeling requirements specified in these Federal regulations and tariffs will apply to all viable recombinant DNA materials in which any portion of the material is derived from an etiologic agent listed in paragraph (c) of 42 CFR 72.25. Additional information on packing and shipping is given in a supplement to the guidelines (Appendix D, part X).

D. Biological containment levels - Biological barriers are specific to each host-vector system. Hence the criteria for this mechanism of containment cannot be generalized to the same extent as for physical containment. This is particularly true at the present time when our experience with existing host-vector systems and our predictive knowledge about projected systems are sparse. The classification of experiments with recombinant DNAs that is necessary for the construction of the experimental guidelines (Section III) can be accomplished with least confusion if we use the host-vector system as the primary element and the source of the inserted DNA as the secondary element in the classification. It is therefore convenient to specify the nature of the biological containment under host-vector headings such as those given below for Escherichia coli K-12.